Role of innate lymphoid cells in allergic diseases

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ABSTRACT

Background: *Over the past decade, there has been increasing interest and research into understanding the type 2 immune responses by the epithelium-derived cytokines interleukin (IL) 33, IL-25, and thymic stromal lymphopoietin. Innate lymphoid cells (ILC) are a unique family of effector immune cells that functionally resemble T cells but lack clonal distributed antigen receptors. Group 2 ILCs, ILC2s, are known for their capability to secrete proallergic cytokines, including IL-5 and IL-13. ILC2s are enriched at mucosal barriers in lung, gut, and skin, and their activation has been associated with a variety of allergic disorders.*

Objective: *To study the role of ILC2 in different allergic disorders, including allergic rhinitis, asthma, atopic dermatitis, and food allergies.*

Methods: *A MEDLINE search was performed for articles that reported on ILC2 in allergic disorders, including allergic rhinitis, asthma, atopic dermatitis, and food allergies.*

Results: *A review of the literature revealed an important role of ILC2 in various allergic disorders.*

Conclusion: *Identification of ILC2s in patients with allergic rhinitis, asthma, and atopic dermatitis indicates that these cells may represent a new therapeutic target. In this review, we discussed the current understanding of ILC2 biology and its function and regulation in various allergic diseases.*

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The discovery of the innate lymphoid cell (ILC) family has greatly expanded our knowledge within the past 8 years. ILCs are unique subsets of lymphocytes that do not express rearranged antigen receptors but transcriptionally and functionally mirror T-helper (Th) cells. ILCs have been categorized into three different groups, *viz.*, ILC1, ILC2, and ILC3, based on their distinct pattern of cytokine production and transcription factors. ILC2s are described in both humans and mice, and can induce a type 2 inflammatory response.[1] ILC2s share many functional similarities with Th2 lymphocytes because they produce type 2 cytokines interleukin (IL) 4, IL-5, IL-9, and IL-13 as well as other effector molecules, *e.g.*, vascular endothelial growth factor (VEGF).[2–10] A subset of IL-25–responsive ILC2s, in addition, produce IL-17 and is termed inflammatory ILC2.[11]

Unlike T cells that recognize specific antigens, ILC2s respond to nonspecific cell-derived factors, such as IL-33, IL-25, and thymic stromal lymphopoietin (TSLP), and to eicosanoids.[2–5,12–15] Both human and mouse ILC2 produce large amounts of IL-5 and IL-13, which promote airway inflammation and hyperresponsiveness. An interesting difference has been observed between mouse and human ILC2 with regard to IL-4 production. Activated mouse ILC2s do not readily secrete IL-4 in response to IL-25 and IL-33, although they may be induced to produce IL-4 by leukotriene D4. However, activated human ILC2 produce large amounts of IL-4, which indicates that human ILC2 might be involved in Th2 priming.[3,13,16] ILC2 numbers are increased in the tissues of patients with various allergic disorders, including allergic rhinitis (AR), asthma, atopic dermatitis (AD), and eosinophilic esophagitis (EoE).[14,17–20] In this review, we summarized the current understanding about the function and regulation of ILCs in various allergic diseases.

ALLERGIC RHINITIS

Classic symptoms of AR include sneezing, rhinorrhea, nasal pruritus, and congestion, caused by an immunoglobulin E (IgE) mediated an early phase response due to allergen exposure.[21,22] A number of studies showed evidence of increased epithelial proinflammatory cytokines in patients with AR. IL-25, IL-33, and TSLP were detected in the nasal lavage from patients with house-dust mite (HDM) sensitivity.[23,24] Furthermore, IL-33 and TSLP messenger RNA (mRNA) levels were high in nasal epithelium of patients with AR.[25–29] IL-33 is thought to cause both IgE-mediated histamine release as well as release of IgE-independent cytokines and chemokines from mast cells.[25] Wang *et al.*[30] showed that allergen stimulation of basophils

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from patients allergic to grass or birch pollen upregulated IL-25 expression in peripheral blood mononuclear cells (PBMC) compared with controls. Moreover, high levels of IL-25R mRNA expression by $CD4^+$ cells in PBMCs from patients with AR were reported.[30,31] This study also demonstrated that cellular apoptosis was inhibited by IL-25 stimulation of basophils and enhanced IgE-mediated degranulation.[30]

There is emerging evidence that proallergic cytokines and ILC2 pathways contribute to the pathogenesis of inflammatory nasal diseases, particularly AR. There is some evidence that the number of ILC2s correlates with the severity of nasal disease, which may have clinical implication in the management of AR, especially in patients resistant to treatment.[32] Doherty *et al.*,[33] demonstrated that the percentage of prostaglandin D2 receptor 2 (CRTH2)-expressing ILC2 is increased in peripheral blood after allergen challenge in patients with cat allergy. Similarly, Zhong *et al.*[34] demonstrated that ILC2 levels were elevated in patients with HDM sensitivity and the level of peripheral blood ILC2 correlated with symptom severity. Interestingly, Fan *et al*.[35] found that the level of ILC2 was elevated in the peripheral blood of patients with AR and with HDM sensitivity compared with healthy controls; however, there was no difference between the ILC2 levels in patients with AR with mugwort sensitivity compared with healthy controls. A possible explanation given by the investigators is that HDM stimulates a stronger immune response to ILC2 than do plant allergens, *e.g.*, mugwort, or a different pathway is used to stimulate an immune response by HDM allergens than by plant allergens. They further showed that there was a significantly greater release of IL-13 and IL-5 from PBMC when incubated with IL-33 with or without IL-25 in the presence of IL-2 in patients with AR and with HDM sensitivity compared with mugwort sensitivity, which suggests that ILC2 plays a role in AR secondary to HDM sensitivity by producing IL-5 and IL-13.[35]

Allergen immunotherapy (AIT), treatment for allergic rhinitis, has been an area of interest to find markers for therapeutic success. Studies in patients responsive to AIT, showed a decreased response from ILC2 with allergen stimulation than healthy controls.[36] Other experiments done by this same group also looked at the role of ILC1 and ILC3 along with ILC2 in AR. Furthermore, the ILC2:ILC1 ratio in patients who responded to AIT was similar to those of healthy controls, which suggested a possible role of ILCs as a biomarker for therapeutic response with AIT in patients with AR. Lombardi *et al.*,[37] found that ILC2 and ILC3 frequencies were significantly increased in grass-allergic patients during the grass pollen season only whereas the frequency of ILC1 did not vary. Furthermore, they showed that sublingual immunotherapy in patients outside of the pollen season did not significantly affect ILC1, ILC2, and ILC3. ILC1 from patients with AR produced less interferon gamma (IFNγ) compared with patients without AR with an *in vitro* stimulation. Whole-transcriptome analysis showed upregulation of murine osteosarcoma viral oncogene homolog (FOS), murine osteosarcoma viral oncogene homolog B (FOSB), and Jun Activation Domain Binding Protein (JUN) genes in ILC2 from patients with AR compared with patients without AR. This indicated that, not only is regulation of ILC2 complex and multifactorial, but there is evidence to indicate differences in regulation of ILC1 and ILC3 between patients with AR and compared with healthy controls.[37]

Kato *et al.*[38] developed a novel mouse model to study localized allergic rhinitis in which they nasally sensitized mice to ragweed. In this murine model, they found ILC2 in the nasal mucosa. They further studied innate immunity in these nasally sensitized mice by using T- and B-cell deficient recombinant activation gene 2 $(Rag2)^{-/-}$ mice.[38] They found that, although there was reduced infiltration of eosinophils at an early time point, there still was some influx of eosinophils compared with a phosphate buffered saline (PBS) control group, which indicated that ILC2 may have a role in early eosinophil infiltration; however, eosinophil infiltration was almost nonexistent at a later time point suggesting ILC2 do not have a role in late eosinophil infiltration in nasally sensitized AR.[38] This data indicates that that T cells may be crucial for mast cell and/or basophil responses and that ILC2 alone may not induce strong localized inflammatory responses; however, ILC2 can induce Th2 inflammation without the acquired immune system. Overall these experiments highlight the role of both the innate immune system and the adaptive immune system in nasal inflammation and responses. Further studies are required to assess the role of ILC2 in non-AR.[38]

ASTHMA

Asthma is characterized by inflammation, airway hyperresponsiveness, and reversible airflow obstruction.[39] It was previously thought that asthma involved mostly Th2 cells and eosinophils; however, recently, it was shown that asthma is more than a Th2 response and is a heterogeneous disorder with several distinct phenotypes.[40] Allergic asthma is triggered by exposure to allergens in a individual who is sensitized, which causes eosinophilic airway inflammation. Nonallergic asthma is associated with exposure to environmental triggers, such as cigarette smoke, diesel particles, ozone, viral infections, exercise, stress, and obesity.[40] This phenotype is often associated with neutrophilic airway inflammation and innate immunity independent of Th2 cells.[41–44]

Asthma is mediated by Th2 cells, which play a critical role in this disease by production of cytokines, such as IL-4, IL-5, and IL-13. In asthma, IL-4 is important for isotype switching, which leads to increased IgE production, whereas IL-5 is important for eosinophil growth and differentiation. IL-13 causes airway hyperreactivity, a hallmark of asthma, by affecting epithelial cells and airway smooth-muscle cells. Several studies show that ILC2s are associated with allergic asthma.[1] The initial studies of ILC2s were performed in murine models.[2,4–5] Subsequent studies indicated that ILC2 has an important role in human respiratory tract. Allakhverdi *et al.*[45] reported that IL-13– and IL-5–producing cells with characteristics of ILC2 cells were present in sputum of patients with asthma compared with healthy controls. They also found that these cells exhibited TSLP and IL-33 receptors, which produced IL-13, IL-5, and chemokines when stimulated by TSLP and IL-33. When specific allergen challenge was performed, the number of ILC2 increased.

In another study, Prefontaine *et al.*[46] reported that, in patients with severe asthma, airway epithelial cells, and airway smooth-muscle cells might be the source of IL-33. Bartemes *et al.*,[17] in a prospective study, investigated whether an ILC2 response can be used as a biomarker of human disease. PBMCs from patients with asthma, patients with AR, and healthy control subjects were cultured *in vitro* with IL-25 or IL-33. They found that innate type 2 responses were increased in patients with asthma compared with patients with AR. Lombardi *et al.*[37] reported greater expression of type-2 cytokines, associated transcription factor, and signaling transduction molecules in ILC2 was found in subjects with atopy and asthma. An increased ILC2 number can be detected in the airways of patients with severe asthma.[47] Furthermore, a high number of IL-5+ and IL-13+ ILC2s has been reported in sputum of patients with severe asthma with persistent eosinophilic airway inflammation, despite treatment with oral corticosteroids.[48]

Chen *et al.*[49] investigated the role of ILC2 in allergen-induced airway eosinophilic responses in subjects with atopy and asthma. They found a significant increase in total IL-5+, IL-13+ ILC2 in the sputum 24 hours after allergen challenge, coincident with a decrease in blood ILC2. The investigators concluded that, although innate and adaptive immune cells are increased in the airways associated with allergic asthma, total and type 2 cytokine-positive ILC2 are increased only within the airways. Saglani *et al.*[50] reported IL-33, a potent activator of lung ILC2, as a factor that regulates airway remodeling in children with severe steroid-resistant asthma. In addition, we found that ILC2 in patients with asthma and upregulated expression of VEGF, which is a potent inducer of airway hyperresponsiveness.[10] Whether inflammatory ILC2s are present in patients with asthma remains unknown and is a topic that warrants further investigation.[11]

There is emerging evidence that asthma may not simply be a Th2-dependent, IgE-mediated allergic inflammatory disease but that it also involves an innate pathway in which ILC2s provide a cellular source of IL-5 and IL-13, important for initiation of adaptive type 2 immune responses.[51–53] Evidence for a role for ILC2s in driving type 2 immune responses comes from animal studies, with little information from patients with inflammatory disease and less so from patients with asthma.[54,55] ILC2s, which are the earliest source of type 2 cytokines, mediate the initiation of eosinophilic inflammation in patients with asthma.[56] Because there is evidence that Th2 cytokines are the product of Th2 cells, a number of treatments have been attempted that target Th2 cells and their cytokine, with modest response.[57–63] It has been hypothesized that IL-5 and IL-13, Th2 cytokines, have been good therapeutic targets, are produced by ILC2s that are constitutively active deep inside mucosal surfaces.[64] Therefore, agents that target ILC2, the source of these cytokines, deep in the mucosal tissues may prove more effective[55]

Barnig *et al.*[65] showed that, similarly to IL-25 and IL-33, prostaglandin D2 (PGD2) stimulated release of IL13 by $CRTH2^+$ ILC2. Furthermore, the ILC2 as well as natural killer (NK) cells expressed receptor A lipoxin/formyl peptide receptor 2 (ALX/FPR2) receptors. The ligand for this receptor, lipoxin A_4, stimulated an anti-inflammatory response by decreasing PGD2 and secretion of IL-13 by ILC2, which suggests a potential role of lipoxin A4 (LXA4) in treating asthma.[65] Sugita *et al.*[66] examined the role of ILC2 in regulating bronchial epithelial tight junction and barrier function in bronchial epithelial cells of patients with asthma, healthy human subjects, and ILC2 deficient mice. They found that when ILC2 was cultured with human bronchial epithelial cells, there was barrier dysfunction, which was represented by decreased transepithelial electrical resistance and increased epithelial permeability for large molecules across the epithelial layers in the experiment.

In subsequent experiments, this barrier dysfunction was reversed when IL-13 was neutralized in cultures of ILC2 and human bronchial epithelial cells. This indicated that ILC2 leads to bronchial epithelial barrier dysfunction through IL-13. Furthermore, in a murine model, IL-33 administration to wild-type mice and $Rag2^{-/-}$ mice, which lack T and B cells, resulted in a disrupted tight junction; however, IL-33 administration to mice that lack ILC2 in addition to T and B cells, did not show any barrier dysfunction. Lastly, when mice were treated with IL-13, there was a significantly lower expression of tight junction mRNA. All together, these experiments revealed the role of ILC2 and IL-13 in bronchial epithelial barrier dysfunction.

ILCs are classically divided into three subsets, ILC1, ILC2, and ILC3, based on their molecular and functional resemblance with effectors T cells. Forkhead Box P3 (FOXP3)-expressing ILCs that resemble regulatory T (Treg) cells have not been identified. Nevertheless, there was recent research of a similar subset that produces IL-10 and requires autocrine Transforming Growth Factor β1 (TGFB1) for maintenance.[67] These cells were termed regulatory ILC (ILCreg). ILCreg can inhibit innate intestinal inflammation. Whether ILCreg are involved in asthma and allergic disease, however, remains unknown. In addition, ILC2s possess substantial functional heterogeneity. A subset of ILC2 also produced IL-10, termed $ILC2_{10}$.[68] Enhanced generation of $ILC2_{10}$ is associated with decreased eosinophil inflammation in mouse models, which indicates a regulatory function of $ILC2_{10}$. Function and regulation of these ILCregs in asthma and allergy warrants future investigation.

ATOPIC DERMATITIS

AD is a chronic pruritic inflammatory skin disease that occurs most frequently in children but that also affects adults. Fillagrin (*FLG*) is an important gene involved in skin barrier function. *FLG* mutation can lead to AD, and studying this gene has helped our understanding of the pathogenesis of AD.[69,70] Although high levels of IL-13 and IL-4 are known to be expressed in AD lesions, little is known regarding how an inherited epidermal abnormality leads to impaired skin barrier and skin inflammation.[14,71] The acute initiation of AD lesions is characterized by increased expression of Th2, Th22, and Th17 cytokines. Th2 and Th22 cytokines (IL-4, IL-13, IL-31, IL-22) seem to modulate the epidermal barrier function by suppressing the expression of terminal keratinocyte differentiation genes (*e.g., FLG*), which inhibits the production of antimicrobial peptides (AMPs) and promotes epidermal hyperplasia.[71,72]

The discovery of ILCs raises the question of their role in the involvement of AD. Maryam Salimi *et al.*[73] examined peripheral blood and acute skin lesions of adults with AD for the presence of ILC2s to investigate whether ILC2 cells might contribute to the pathogenesis of AD. They found significantly more ILC2s in the skin biopsy specimens in the patients with AD than in healthy controls, but they found no difference in frequency of ILC2 in the peripheral blood of patients with AD compared with control patients. Kim *et al.*[14] demonstrated that ILC2s accumulate in the lesional skin of patients with AD and that depletion of murine ILCs significantly ameliorates AD-like inflammation in their model, which supports the role of ILC2s in skin inflammation. Furthermore, they showed that ILC2 responses in the skin were dependent on TSLP. Targeting of TSLP in the future may provide a therapeutic option for a certain group of AD.

FOOD ALLERGY

Food allergies are common and affect up to 10% of the population, with an increasing prevalence over the past 2 to 3 decades, particularly in industrialized countries.[74] Food allergy is thought to present secondary to a breakdown of immunologic and clinical tolerance to an ingested food, which results in IgE-mediated reactions or non–IgE-mediated disorders. This breakdown of tolerance leads to food allergen sensitization, which commonly occurs in the gastrointestinal tract and/or skin, presumably in conjunction with inflamed barrier function.[75,76] Immune tolerance occurs in the gastrointestinal tract through the presentation of food antigen by $CD103^+$ dendritic cells (DC), whereas, in the skin, tolerance develops through presentation of food antigen by $CD11b^+$ dermal DCs and Langerhans cells.[77] DCs and Langerhans cells are antigen-presenting cells that induce Treg cells in the mesenteric and regional lymph nodes to promote tolerance. Induction of Treg cells is believed to be compromised in patients with food allergy and replaced by generation of unique antigen-specific Th2 cells that drive IgE class switching.[78] Production of Th2 cytokines IL-4, IL-5, and IL-13 amplifies the allergic response by blocking induction of allergen-specific Treg cells and causes Th2 cell differentiation and IgE-driven mast cell activation.[79,80]

Although there are conflicting reports on the role of ILC2 in food allergen sensitization, numerous studies, mostly in murine models, seem to indicate that ILC2 plays a role in food allergy.[81,82] In murine models, oral feeding of antigen plus adjuvant stimulates gut epithelial cells to express IL-33, which promotes the Th2 response.[81] It has been reported that IL-33 promotes food allergy through expansion and activation of ILC2s, which then respond by producing large amounts of IL-4 and leads to suppression of Treg cells in the skin, lung, and small intestine.[79] IL-33 also contributes to acute reactions to food by acting directly on mast cells and enhancing IgE-mediated activation.[76] Noval Rivas *et al.*[83] demonstrated that ILC2s play an important role in mediating oral sensitization to food allergens in a murine model, and, interestingly, mice prone to food allergy did not develop sensitization when ILC2 differentiation was compromised by concurrent IL-33R deficiency. The investigators report that ILC2s produce copious amounts of IL-4 sufficient to suppress allergen-specific induced-Treg (iTreg) cell differentiation during food allergy.[83] Burton *et al.*[84] reported intestinal ILC2 expansion in food allergy, driven by IgE-activated mast cells, which can enhance mast cell mediators of anaphylaxis production.

Table 1 **The relative frequency of inflammatory cells in known and projected subtypes of esophagitis**

	Controls	EoE	LyE	High Cells, Mixed	Low Cells, Mixed	Group 2 ILC Esophagitis+
Eosinophils	0	****	0	***	**	?
Adaptive Lymphocytes	*	***	****	***	**	?
Group 2 ILC	*	**	?	?	?	?

*EoE = Eosinophilic esophagitis; LyE = lymphocytic esophagitis; ILC = innate lymphoid cell. *Relative presence of cells (0 -****) extracted from data in 3 different studies. (References 89, 90, 19)*

** Asterisks indicate the relative presence of cells extracted from three different studies.*

+ Speculated phenotype.

EOSINOPHILIC ESOPHAGITIS

The medical history of EoE since its original description in 1978 was recently reviewed.[85] Well recognized as a complex allergic disease by pediatric and adult medical specialists, its prevalence is increasing.[86] A recent update summarized the histologic description of EoE, which includes the standard description of eosinophilic infiltration but also mentions increases in mast cells, B cells, and IgE-containing cells.[87] T-cell infiltration in EoE has not been historically discussed in the context of EoE histopathology. A separate esophageal entity, lymphocytic esophagitis (LyE), now well described in the adult literature, was first described in 2006.[88] It is histologically described as having peripapillary intraepithelial lymphocytes with few or no granulocytes.

In any normal esophageal biopsy specimen, lymphocytes are acceptably found and number $\leq$ 10/hpf.[88] LyE is typically diagnosed with lymphocytes $\geq$ 20/hpf (variable among reports).[88] Several recent studies seem to have expanded the concept of eosinophilic (granulocytic) and/or lymphocytic infiltration(s) in inflammatory esophagitis. The first report, published in 2017, presented the pathologic (histologic) pattern of eosinophilic and lymphocytic involvement in a collection of subjects (N = 311).[89] The investigators, who also originally described LyE in 2006, used the T-cell marker CD3 coupled with the presence of granulocytic cells (hematoxylin and eosin stain) to classify their results. They divided their findings into four subgroups: EoE, LyE, compound EoE-LyE, and lymphocytic infiltration. The cell marker used to identify lymphocytes was CD3, and, because type 2 ILCs do not express CD3, the relative percentage of ILCs in their four phenotypes was not determined. Both adults and children were included, and children (ages < 20 years) were identified in all four phenotypes.

In a second report, another medical center recently published their efforts to use cellular immunochemistry staining, coupled with flow cytometry, as a potential replacement of standard histologic assessment of EoE.[90] Adaptive lymphocytes were present in both controls and subjects with active EoE, although intraepithelial lymphocytes were significantly higher in the subjects with active EoE. A third, collaborative project in the United States used subjects with active EoE and controls to compare the presence of ILC2s in esophageal biopsy specimens.[19] ILC2s lack the markers for all other known immune cell lineages but express the chemoattractant homologous molecule seen on Th2 lymphocytes ($CD45^+$ Lin^- $CRTH2^+$). Although controls demonstrated small numbers of adaptive lymphocytes with limited group 2 ILCs, subjects with active EoE had a significantly higher number of adaptive and innate lymphocytes (ILCs).

Finally, the collaborators who identified ILC2 in EoE expanded their findings in a preliminary report.[91] They identified a key cytokine, IL-9, in regulating ILC2 biology and found that ILC2 from patients with active EoE upregulated IL-9R expression.[91] We compiled information from recently published studies to construct a tabular review of inflammatory esophagitis (Table 1). The relative presence of the constitutively present or increased inflammatory cell population indicates the strength of the literature evidence for the cells (present in esophageal tissue) that might contribute to the pathology of different esophageal inflammatory subtypes. The increasing prevalence of EoE has mirrored the increase in all allergic diseases in the past 3 decades.[85] Our understanding of the pathophysiology of EoE has made quantum leaps in a short period of time, with techniques learned from asthma and AD research. Analysis of the limited but current data strongly indicated a role for the ILC2 cell type in EoE, which mirrors findings in other allergic diseases. Furthermore, the role of ILCs in LyE, and, potentially, in other forms of inflammatory esophagitis, awaits additional study. The role of eosinophils and ILC in gastrointestinal inflammation was recently reviewed.[92,93]

CONCLUSION

Intensive work has been performed to reveal the function and regulation of ILC2s in allergic diseases, particularly AR and asthma, in recent years. Still, there

is much more to investigate about this unique type of immune cell. What are the microanatomic locations of ILC2s in the upper and lower airways and in the skin? What other immune and nonhematopoietic cells directly and indirectly interact with tissue-resident ILC2s? Do ILC2s participate in forming specialized microanatomic structures that are yet to be characterized, and if so, how? What signals activate ILC2s in human allergic diseases, and how do such signals differ from those involved in T-cell activation? With the advance of new techniques and the increasing awareness of innate lymphocytes in health and disease, these and related questions, it is hoped, will be resolved in the very near future.

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